Antifungal activity of the essential oil of Eugenia caryophyllata on Candida albicans, Aspergillus niger and Aspergillus flavus

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ABSTRACT

The composition of the essential oil of Eugenia caryophyllata and its antifungal activity on Candida albicans, Aspergillus niger and Aspergillus flavus fungal strains were studied in Iran. Essential oil from the flowers parts of the plant was obtained by hydrodistillation and analysed by GC and GC-MS. The oil showed high contents of Eugenol, B-caryophyllene and Euguggenyl acetate. The MIC was used to evaluate the antifungal activity against Candida albicans ATCC 10231, Aspergillus niger ATCC 9642 and Aspergillus flavus ATCC 9643. Antifungal activity was evaluated for the essential oil and simultaneously for Amphotericin B. Results showed that Eugenia caryophyllata essential oil exhibited a significant activity against fungi, and its MIC on Candida albicans, Aspergillus niger and Aspergillus flavus were respectively 0.50, 0.125 and 0.25 µg ml⁻¹ (ppm). The present study indicates that Eugenia caryophyllata essential oil has considerable antifungal activity, deserving further investigation for clinical applications.

Key words: Eugenia caryophyllata, MIC(minimal inhibitory concentration), Antifungal activity.

INTRODUCTION

Fungal infections have been increasing in recent years due to a growing number of high-risk patients, particularly immunocompromised hosts. Candida is the third- or fourth-most-common isolate in nosocomial bloodstream infections in the USA. In addition, The mortality rate due to invasive aspergillosis increased by 357% between 1980 and 1997 in the USA. In spite of the introduction of new antifungal drugs, they are limited in number. The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies (Eugenia pinto, 2006).

Some essential oils show an important antifungal activity against yeasts, dermatophyte fungi and Aspergillusstrains, which could predict therapeutic benefits, mainly for diseases with mucosal, cutaneous and respiratory tract involvement. (Pina-Vaz et al., 2004; Salgueiro et al., 2003, 2004),
The objective of our present research was to evaluate the antifungal activity and investigate the mechanism of action of *Eugenia caryophyllata* oil.

**METHOD**

**Essential oil analysis**

Essential oil from the flowers of the plant was obtained by hydrodistillation and analysed by GC and GC–MS. Gas chromatography (GC) and GC–mass spectrometry (MS) analysis of essential oil of clove were performed. The oil showed high contents of Eugenol, B-caryophyllene and Eugenyl acetate.

**Plant material and chemicals**

The plants were collected from Tokestan, 11<sup>th</sup> Km. Gorgan-Mashhad Road, Gorgan, Iran.

**Essential oil analysis**

Essential oil was isolated by water distillation for 3 h from air-dried material, using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia (Council of Europe, 1997).

**Gas chromatography (GC–FID)**

Gas chromatography–mass spectrometry was performed on a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with flame ionization detector and capillary column HP-101 (Methyl silicone fluid), 25 m x 0·2 mm i.d., coating thickness 0·2 µm. Chromatographic conditions were as follows: helium as carrier gas at 1·0 ml min<sup>-1</sup>; injector and detector temperatures, 250°C and 300°C. Oven temperature was isothermal at 70°C for 2 min, then increased to 200°C, at a rate of 3°C min<sup>-1</sup> and held isothermal for 15 min. Volume injected 1µl. Split ratio 1 : 50. MS conditions: ionization voltage: 70 eV; ion source temperature: 280°C; mass range: 30–300 mass units.

**Isolation and detection of fungi**

The antifungal activity of the essential oil was evaluated against *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 9642, and *Aspergillus flavus* ATCC 9643. The fungal isolates were identified by standard microbiology methods and stored in Sabouraud dextrose broth with glycerol at -70 °C.

Fungi were plated on Sabouraud 2% (w/v) glucose agar (SGA), and incubated at 25 ± 2°C for the 5–7 days.

**Antifungal activity testing by dilution method**

For the antifungal activity testing, clove essential oil were dissolved in 96% (v/v) ethanol and then diluted with 30% (v/v) ethanol in distilled water with 0·1% (w/v) Tween 80. Final concentrations of clove essential oil was 2% (w/v).

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were
determined by using the serial broth dilution method described by Pepeljnjak et al. (2003). Serial twofold dilutions in DMSO, ranging from 0.02 to 20 µl ml–1, were tested for essential oil. In addition, the reference antifungal compounds, fluconazole (Pfizer) for Candida albicans or amphotericin B (Sigma) for Aspergillus, were used as standard antifungal drugs. Twofold serial dilutions ranging from 0.25 to 128 µg ml–1 for fluconazole and 0.016 to 16 µg ml–1 for amphotericin B were used. Quality control determinations of the MICs of fluconazole and amphotericin B were performed by testing C. parapsilosis ATCC 90018 and C. krusei ATCC 6258. The results obtained were within the recommended limits.

MIC is defined as the lowest concentration of extract or essential oil that allows no more than 20% growth of the fungus, visualized as a reduced number of colonies after removing the loop with approx. 10 µl of each dilution, and then inoculated on SGA and incubated at 25 ± 2°C for 7 days. MFC is defined as the lowest concentration of essential oil that completely inhibited the growth of fungi. These experiments performed in duplicate were repeated independently three times and yielded essentially the same results. A range of values is presented where different results were obtained. Two growth controls, RPMI medium and RPMI with 2.0 % (v/v) DMSO, were included for each strain.

Statistics: The data obtained as MIC and MFC of essential oil, expressed in µg ml–1, were statistically analysed by using the Wilcoxon matched pairs test. The level of P < 0.05 was considered statistically significant.

Table 1. Antimicrobial activity (MIC) and (MFC) of the essential oil of the Eugenia caryophyllata for Candida albicans, Aspergillus niger and Aspergillus flavus

<table>
<thead>
<tr>
<th>Candida albicans</th>
<th>Aspergillus niger</th>
<th>Aspergillus flavus</th>
<th>Eugenia caryophyllata effects</th>
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<tr>
<td>0.50</td>
<td>0.125</td>
<td>0.25</td>
<td>MIC in µg ml–1</td>
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<tr>
<td>0.50</td>
<td>0.25</td>
<td>0.30</td>
<td>MFC in µg ml–1</td>
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Results were obtained from three independent experiments performed in duplicate.

RESULTS AND DISCUSSION

The oil was obtained from air-dried plant material in a yield of 1.8 % (v/w). Evaluation of MIC showed that the oil was active against all the tested strains (Table 1). Eugenia caryophyllata essential oil exhibited significant antifungal activity. MIC values ranged from 0.125 to 0.25 µl ml–1 against Aspergillus strains. Candida showed the highest MIC values, 0.50 µl ml–1. It is difficult to attribute the activity of a complex mixture to particular constituents. The importance of the phenolic hydroxyl groups for the antimicrobial activity of the monoterpenoids has previously been reported (Adam et al., 1998; Aligiannis et al., 2001; Dorman & Deans, 2000; Nostro et al., 2004; Sivropoulou et al., 1996).

In conclusion, the findings of the present study indicate that Eugenia caryophyllata essential oil has potential as a topical antifungal agent against fungi that are pathogenic to humans. This essential oil is a broad-spectrum agent that inhibites Aspergillus and Candida species. Given the results described above, particularly the possible mechanisms of action, which might induce side-effects in humans, these antifungals require further investigation.

The results presented should stimulate studies on toxicity, improved formulations and the determination of optimal concentrations for clinical applications, as well as comparative studies alongside currently used drugs of the therapeutic efficacy of essential oils to control infections.
REFERENCES


